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Identification, purification and characterization of a streptococcal protein antigen with a molecular weight of 3800

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Summary. A small molecular weight streptococcal antigen of about 3800 was isolated from *Streptococcus mutans*. The peptide was obtained by gel filtration of a predominantly 185,000 mol. wt. antigen preparation, with two major antigenic determinants (I/II), on Sephacryl S-200, in the presence of sodium dodecyl sulphate (SDS). The 185,000 mol. wt. antigen was prepared from the culture supernatant of *S. mutans* by ammonium sulphate precipitation, DEAE cellulose chromatography and gel filtration on Sepharose 6B. The 3800 mol. wt. material gave a single band on SDS/polyacrylamide gel and reacted with antisera to streptococcal antigen I/II, I and II but not III. Furthermore, it was digested by pronase, contained only traces of carbohydrate and lipids were not detected. It is suggested that SA I/II is either synthesized in a range of molecular sizes from 185,000 to 3800 or the former is broken down by streptococcal proteases into smaller fragments.

INTRODUCTION

The antigenic components of *Streptoccocus mutans* have been studied extensively because of the importance of this organism in the development of dental caries (Hamada & Slade, 1980). Protein antigens have been of particular interest; glucosyl-transferases have been described early (Carlsson, Newbrun & Krasse, 1969; Guggenheim & Newbrun, 1969) and dextran-binding protein more recently (McCabe, Hamelik & Smith, 1977). Four predominantly protein antigens (I to IV) have been identified in the culture supernatant of *S. mutans* on immunodiffusion and on immunoelectrophoresis against the corresponding antisera (Russell & Lehner, 1978). Glycoproteins with similar molecular weights (mol. wts) of 190,000 were isolated by other workers (Hardy *et al.*, 1981; Russell M.W., 1979) and another glycoprotein with a mol. wt. of 29,000 was reported (Russell R.R.B., 1979). Further investigations showed that antigen I and II are usually associated in a single molecule (185,000), as an important cell surface antigen (Russell *et al.*, 1980a; Zanders & Lehner, 1981). Antigen II (48,000) can be separated and isolated by pronase digestion of antigen I/II, followed by column chromatography (Russell *et al.*, 1980a). Antigen I (150,000) can be isolated by affinity chromatography (Russell *et al.*, 1980b).

In an attempt to break down the relatively large 185,000 mol. wt. streptococcal antigen (SA) I/II into smaller fragments which would still retain their antigenic properties, a variety of enzymes have been used (Russell *et al.*, 1980a; Zanders & Lehner, 1981; A. S. M. Giasuddin & T. Lehner, unpublished). It became evident, however, that if the 185,000 mol. wt.

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SA I/II preparation is overloaded on 15% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), then small amounts of low mol. wt. materials can be found which react with anti-streptococcal antisera. The object of this paper is to describe the analytical and preparative methods leading to the isolation and characterization of a protein of small molecular weight (3800), from the protein of high molecular weight (185,000). Both the small and large streptococcal protein preparations share the antigen I and II determinants.

MATERIALS AND METHODS

Organism and growth conditions

For the purification of antigens, *Streptococcus mutans* (serotype c; Guy's strain) was grown in 12 litres of pre-warmed Todd-Hewitt broth (Oxoid) at 37°, using an overnight culture in 100 ml of Todd-Hewitt broth as an inoculum. The growth was continued for 60–69 hr. The culture supernatant was separated by centrifugation in a continuous flow rotor at 37,000 *g* (Russell *et al.*, 1980a).

Extraction and purification of streptococcal antigen I/II

The material was prepared with some modifications, as described previously (Russell *et al.*, 1980a; Russell & Lehner, 1978). Briefly, the protein antigens in the culture supernatant were precipitated with 75% ammonium sulphate. The precipitate was centrifuged, dissolved in urea–tris buffer, dialysed against water and chromatographed on a diethylaminoethyl (DEAE)–cellulose column (Whatman DE52; 30 × 1·5 cm). The material was eluted from the column with 6 M urea–0·01 M tris buffer (pH 8·0), containing 0·05 M sodium chloride and the fractions were tested in a single radial immunodiffusion (SRID) against antiserum to SA I/II. The fractions that reacted with the antiserum to SA I/II were pooled, dialysed against water, lyophilized and then dissolved in 1% ammonium bicarbonate and gel filtered on Sepharose 6B column (Pharmacia, Great Britain, Ltd.; 90 × 2·5 cm) with the same buffer. The eluate was monitored at 280 nm and 3 ml fractions were tested by SRID against antiserum to SA I/II. The fractions that reacted with the antiserum to SA I/II were pooled and lyophilized.

Polyacrylamide gel electrophoresis

High mol. wt. materials were detected by SDS-PAGE on 7·5% polyacrylamide gels in a vertical slab gel apparatus as described previously (Russell *et al.*, 1980a). Low mol. wt. antigens were detected by SDS-PAGE in the presence of 6 M urea (Bethesda Research Laboratories, U.S.A., Biologue 1981). The 15% resolving gel contained 0·1 M sodium phosphate pH 7·2, 0·1% SDS, 0·02% sodium azide and 6 M urea. A 7·5% polyacrylamide stacking gel in the same buffer was used. Gels were run overnight at 70 V and stained with Coomassie Brilliant Blue. A pre-stained protein mol. wt. standard mixture (BRL, U.S.A.), containing ovalbumin (43,000), α-chymotrypsinogen (25,700), β-lactoglobulin (18,400), lysozyme (14,300), cytochrome c (12,300), bovine trypsin inhibitor (6200) and insulin A and B chains (3000), was used to determine the mol. wts. of the low mol. wt. antigens.

Purification of low mol. wt. antigens

Elution from polyacrylamide gel. A sample of 2·5 mg per gel of the purified antigen I/II was loaded, after equilibration with the sample buffer, on 15% polyacrylamide–6 M urea gel and electrophoresed as described in the earlier section. After electrophoresis, the gel was sliced into five sections according to the mol. wt. ranges (1) >43,000, (2) <43,000 >25,700, (3) <25,700 >18,400, (4) <18,400 >6200 and (5) <6200. The slices were minced separately by forcing them through a hypodermic syringe and the materials were then extracted three times with 0·01 M tris–HCl buffer (pH 8·0), containing 0·05% SDS and 1 mM PMSF (phenyl methyl sulphonyl fluoride), at 37° over 36–48 hr. The three extracts from each slice were pooled, passed through glass fibre filter under vacuum, dialysed extensively against water at 4° and lyophilized. The dialysis tubing used had a mol. wt. cut off of 1000 (Raven Scientific Company Ltd., U.S.A.). The lyophilized material was reconstituted in 0·85% NaCl, centrifuged for 3 min at 25,000 *g* and the supernatant was collected.

Purification by gel filtration. Gel filtration of SA I/II was carried out on a column of Sephacryl S-200 (88 cm × 1·6 cm) and equilibrated with 0·1 M tris–HCl (pH 8·0), containing 4·0% SDS and 0·02% sodium azide. The column was calibrated using α-chymptrypsinogen A (25,700) soyabean trypsin inhibitor (21,500), ribonuclease A (13,700), insulin (6000), insulin A chain (2300) and glutamyl-glycyl-phenylalanine (352). A sample of the starting antigen I/II (6–10 mg) was dissolved in 1·5 ml of the elution buffer, and boiled for 10 min before it was loaded on to the column. The effluent was monitored continuously at 254 nm and 1·5

ml fractions were collected. The absorption of each fraction was also measured at 230 nm. Fractions were pooled over the following mol. wt. ranges: (1) >33,000, (2) 33,000–21,500, (3) 21,500–13,700, (4) 13,700–8000, (5) 8000–3000, (6) 3000–2300 and (7) <2300.

The pooled fractions were dialysed in tubing, with a molecular weight cut off of 1000, in three steps as follows: (i) against two or three changes of water, at room temperature, for 24 hr; (ii) against 40% methanol for 48 hr, with a change at 24 hr; and (iii) against two or three changes of water again for 48 hr. The fractions were then lyophilized and reconstituted in 1·0 ml of 0·85% NaCl. The reconstituted materials were centrifuged for 3 min at 25,000 *g* and the supernatants were collected.

Antisera and serological methods

Antisera were raised in New Zealand white rabbits by intramuscular injections of 1 mg of antigen in Freund's complete adjuvant, followed 3 weeks later by subcutaneous injection of 1 mg antigen in Freund's incomplete adjuvant. Blood was taken 3 or more weeks after the last immunization (Russell *et al.*, 1980a). Single radial immunodiffusion was used for routine detection of antigens in the fractions, in 1·0% agarose gel (Russell & Lehner, 1978), containing 1–2% antiserum in veronal buffer (pH 8). For greater sensitivity the solid phase radioimmunoassay technique was also used (Smith & Lehner, 1981). Protein concentration was estimated by the method of Lowry *et al.* (1951).

Proteolytic digestion of the antigen

Forty-two micrograms of the 3800 mol. wt. antigen was taken up in 600 μl of 0·1 M-tris/HCl and incubated at 37° with pronase (enzyme:protein, 1:100, w/w). Samples of 100 μl were taken at 0 min, 15 min, 30 min, 2 hr, 4 hr and 6 hr and boiled for 10 min. Samples were analysed for antigen I and II determinants by the solid-phase radioimmunoassay technique (Smith & Lehner, 1981).

Carbohydrate analysis

The total monosaccharide contents of the 3800 mol. wt. material and the 185,000 mol. wt. material were determined by the method of Clamp (1974) using a Pye 204 gas chromatograph. A column (1·5 m × 4 mm) was packed with 10% SE-30 (w/v) on Chromosorb W HP AWDMCS, mesh size 100–120. D(−) arabinose was used as standard.

Analysis of lipid content

Total lipid was extracted from 100 μg of the 185,000 mol. wt. material and from 40 μg of 3800 mol. wt. material using chloroform and methanol (2:1, v/v) (Folch, Lees & Stanley, 1957). The extracts were analysed by thin layer chromatography on plates coated with silica gel H containing ammonium sulphate (Kaulen, 1972). Plates were developed with hexane, diethyl ether and acetic acid (60:30:1, v/v) until the solvent front reached the top of the plate. After thorough drying lipids were detected by exposure to iodine vapour for 3–5 min. The following mammalian lipids were used as standards: phospholipid, cholesterol, free fatty acid, triglyceride and cholesterol ester.

RESULTS

Identification of low molecular weight antigens

The starting SA I/II material was examined by sodium dodecyl sulphate/polyacrylamide gel electrophoresis in the presence of 6 M urea. When the gel was overloaded (280 ± 30 μg protein/cm slot), a number of minor components were detected (Fig. 1) with mol. wts lower than that reported for antigen I/II (Russell *et al.*, 1980a). A low mol. wt. component was detected in 27 out of 29 preparations of the starting SA I/II, with a mol. wt. which varied from 3800 to 4500.

Elution of antigens from 15% polyacrylamide–6 M urea gel

The proteins eluted from the 5 slices were assayed for their protein content and antigenicity with antisera to SA I/II, I, II and III by single radial immunodiffusion (Table 1). SA I/II was present in all fractions, including the lowest mol. wt. fraction (fraction 5). A variable amount of SA III (Russell & Lehner, 1978; Russell M.W., 1979) was also detected in fractions 1, 2 and 3, but not in 4 or 5.

Separation by Sephacryl S-200 column chromatography

We have established in preliminary experiments that fractions from the column absorb more strongly at 230 nm than at any other wavelength in the ultraviolet spectrum (Fig. 2). The optical density of the eluted fractions, monitored at 254 nm, were then measured at 230 nm. The fractions were pooled into seven batches

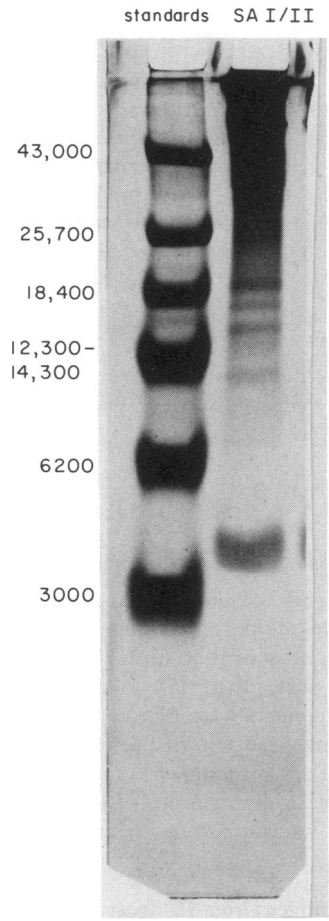

Figure 1. Polyacrylamide gel electrophoresis of 280 μg of the streptococcal antigen I/II (SAI/II) on 15% gel with 6 M urea and 0·1% SDS. *Left-hand lane*—mol. wt. markers: ovalbumin 43,000; α-chymotrypsinogen A 25,700; β-lactoglobulin 18,400; lysozyme 14,300; cytochrome C 12,300; bovine trypsin inhibitor 6200; insulin chain A and B 3000. The gel was stained with Coomassie Brilliant Blue.

according to the mol. wt. ranges, as determined by calibration of the column. Fractions 1, 5 and 6 corresponded to individual peaks, fraction 2 was a shoulder on the major peak of fraction 1, and fraction 3 corresponded to the remainder of the peak (Fig. 2). Fractions 4 and 7 showed minimal absorption. Each of these fractions was then dialysed, lyophilized, reconstituted in normal saline and the protein content was estimated (Lowry *et al.*, 1951). They were then tested for efficiency of separation on both 15% polyacrylamide–6 M urea and 7·5% polyacrylamide gels as described earlier. Fraction 5 (mol. wt. 8000–3000) showed a single band corresponding to a mol. wt. of about 3800 as calculated from the gel (Fig. 3). Fractions 6 and 7 also showed bands equivalent to a mol. wt. less than 4000 but the protein concentrations were less than that in fraction 5 (Table 2).

Single radial immunodiffusion tests showed that SA I and II were present in all seven fractions and that SA III was absent in all but fraction 2, with a trace in fraction 3 (Table 2). The presence of SA I and II but not III in fractions 5, 6 and 7 was confirmed by the solid phase radioimmunoassay. However, all 6 Sephacryl column separations yielded the 3800 d SA in fraction 5 but this material was detected in only 4/6 fractions 6 and 7.

We have also found that when 0·1% SDS was used instead of 4% SDS in the elution buffer, all but fractions 1 and 7 contained the 3800 mol. wt. SA I/II, with increasing intensity of staining from fraction 2 to 6. However, with the 0·1% SDS the higher molecular weight proteins were not separated completely. In contrast, with 4% SDS the low mol. wt. peptide was found only in fractions 5 and to a lesser extent in 6 and 7. It appears then that the 3800 mol. wt. antigen is tightly bound to the high mol. wt. antigen (185,000), along with other proteins of intermediate mol. wts which are demonstrable on the 15% polyacrylamide–6 M urea gel.

Proteolytic digestion of the antigen

Both antigen I and II components of the 3800 mol. wt. peptide were partially digested by 15 min and completely digested by 30 min. The radioimmunoassay showed 1·46% binding with SA I/II antiserum before and 0·63 after 15 min and 0·22% after 30 min digestion. Comparable results were found with SA I and SA II antisera.

Analysis of total carbohydrate content

The 3800 mol. wt. peptide identified in fraction 5 was analysed and compared with the starting 185,000 mol. wt. material. Two samples of the 3800 peptide contained 1·64% and 2·08% (w/w) of monosaccharide, in contrast to a mean (±SD) of 6·57 (±1·63)% of 10 samples of the 185,000 material.

Total lipid content

Thin layer chromatography of 100 μg of the 185,000

Table 1. Protein content and antigenic determinants in the five fractions eluted from the 15% polyacrylamide–6 M urea gel loaded with the streptococcal antigen I/II

Quantity of SA I/II loaded (mg)	Fraction No.	Mol. wt.	Protein content		Antisera to antigens		
			μg	% Yield	I	II	III
5·0	1	>43000	1330	26·60	+	+	+
	2	<43000 >25700	190	3·80	+	+	+
	3	<25700 >18400	178	3·05	+	+	Trace
	4	<18400 >6200	126	2·52	+	+	−
	5	<6200	198	3·96	+	+	−
5·7	1	>43000	1230	24·60	+	+	+
	2	<43000 >25700	180	3·60	+	+	+
	3	<25700 >18400	165	3·30	+	+	Trace
	4	<18400 >6200	110	2·20	+	+	−
	5	<6200	180	3·60	+	+	−
6·5	1	>43000	1211	24·22	+	+	+
	2	<43000 >25700	152	3·04	+	+	+
	3	<25700 >18400	158	3·16	+	+	Trace
	4	<18400 >6200	106	2·12	+	+	−
	5	<6200	157	3·14	+	+	−

(+) Presence of the antigenic determinant.
(−) Absence of the antigenic determinant.

material revealed the presence of free fatty acids, triglycerides and cholesterol esters, with R_f values slightly different from the mammalian lipid standards (Table 3). A spot was found at the origin which was slightly more intense than that found in the blank control, suggesting that traces of phospholipids might be also present. The lipid extract from 40 μg of the 3800 peptide gave only a spot at the origin, comparable with that in the blank and therefore the antigen was considered to be free of lipids.

DISCUSSION

Four SA were identified in extracts of cells and culture supernatants of *S. mutans* (Russell & Lehner, 1978). Three of these antigens have been isolated and characterized as predominantly protein in nature. SA I/II with a molecular weight of 185,000 (Russell *et al.*, 1980a) consists of the two major antigenic determinants I (150,000) and II (48,000). SA I can be isolated by affinity purification (Russell *et al.*, 1980b) and SA II by pronase digestion of SA I/II (Russell *et al.*, 1980a). Separation of SA I/II from *S. mutans* culture supernatant by ammonium sulphate precipitation, followed by ion exchange chromatography and gel filtration resulted in a single band, when 10 μg was applied to 7·5% PAGE containing SDS.

We have now observed that loading the gel with 280 ± 30 μg of this material and using 4% SDS revealed a large number of minor bands between 185,000 and 43,000 on 7·5% gels and between 43,000 and 3800 on 6 M urea, 15% gels. Only the 3 smallest molecular weight bands were examined for the presence of any of the three antigens under investigation. Antigen I/II but not III was found by the corresponding antiserum in the fractions eluted from the gels, at the mol. wt. ranges of 14,000–18,000, 8000–12,000 and about 3800. This suggested that the 185,000 molecule may contain repeating I/II antigenic determinants, of which the smallest unit is about 3800. The existence of SA I/II, probably in a large range of mol. wts from 185,000 to 3800 which were isolated from the culture supernatant of *S. mutans*, can be explained either by the synthesis of SA I/II in a range of molecular sizes or by the breakdown of the 185,000 molecule by streptococcal proteases inside the cells or in the extracellular fluid.

Although the 3800 material could be eluted from the gel, boiling the starting SA I/II in 0·1 M tris–HCl containing 4% SDS for 7 min, and then sieving

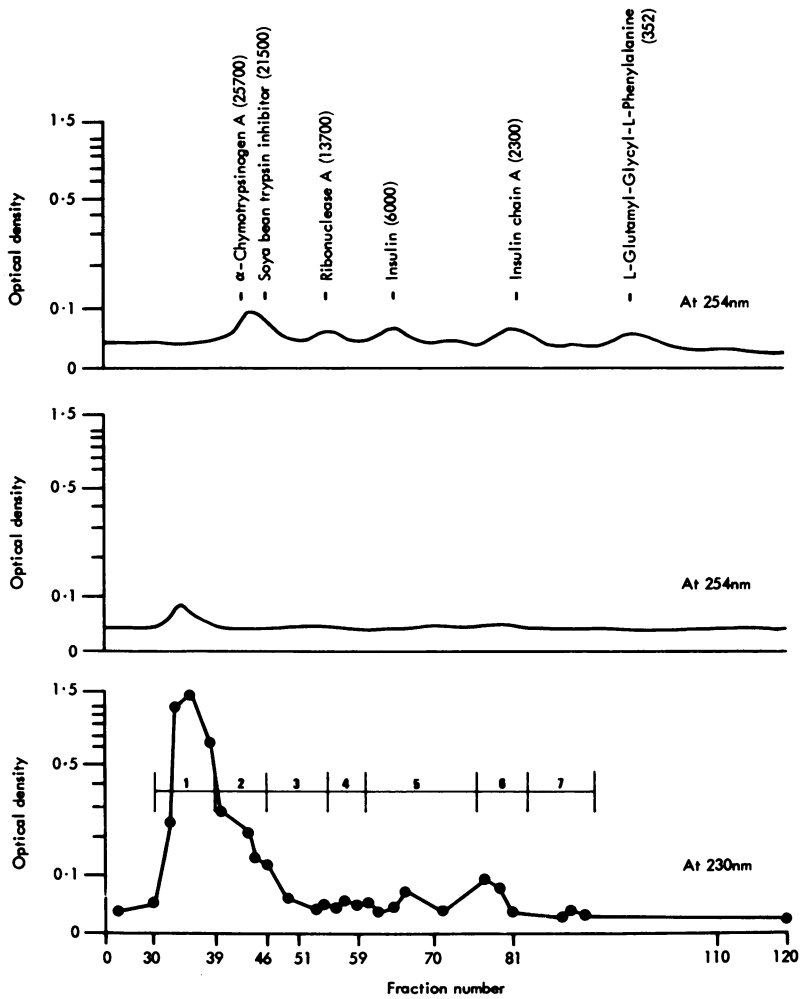

Figure 2. A tracing of the absorption at 230 nm and 254 nm of the individual fractions (1·5 ml) resulting from elution of 10·0 mg of the starting antigen SAI/II from Sephacryl S-200 column. *Upper tracing*, standard mol. wt. markers at 254 nm, *middle tracing*, absorption of the fractions at 254 nm; *lower tracing*, absorption of the fractions at 230 nm.

through Sephacryl S-200 with the same SDS buffer, resulted in a single band of 3800. The presence of both antigen I and II determinants was established by antisera raised against the macro-molecular SA I/II, I and II by both radial diffusion and radioimmuno-assay. SA III was not found by either assay, so that the 3800 SA I/II was free of SA III. Furthermore, the precipitation reaction with the denatured antigen, caused by boiling with 4% SDS buffer, suggests that the three-dimensional antigenic structure must have been restored to that resembling the starting material. However, pronase digested both antigen I and II determinants of the 3800 peptides within 30 min, unlike the 185,000 material in which SA I was readily digested but SA II remained intact even after 16 hr digestion (Russell *et al.*, 1980a). This raises the possibility that in the small molecule both antigens I and II are exposed to the enzyme action, whereas in the large molecule antigen II may be hidden. The yield of the 3800 antigen from 9·3 to 10·1 mg of the starting material varied between 128 and 385 μg (or 1·3 to 3·9%), so that it is a minor component which needs careful handling to obtain adequate amounts for analytical work.

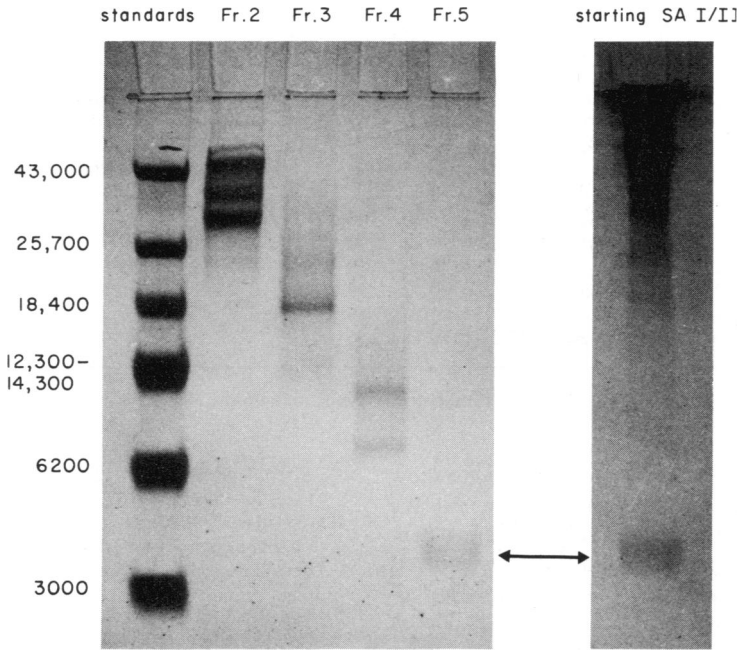

Figure 3. A 15% polyacrylamide–6 M urea gel of fractions 2 to 5 obtained by gel filtration of 10·0 mg of the 185,000 SAI/II antigen on Sephacryl S-200 column. This is compared with the starting SA I/II material.

Table 2. Protein content and antigenic determinants in the seven fractions separated by Sephacryl S-200 column chromatography and showing a range of molecular weights from > 33,000 to < 2300

Fraction No.	Mol. wt.	Protein content		Single radial diffusion with antisera to SA			
		μg	% Yield	I/II	I	II	III
1	> 33000	2031	20·31	+	+	+	−
2	< 33000 > 21500	650	6·50	+	+	+	+
3	< 21500 > 13700	210	2·10	+	+	+	Trace
4	< 13700 > 8000	152	1·52	+	+	+	−
5	< 8000 > 3000	138	1·38	+	+	+	−
6	< 3000 > 2300	64	0·64	+	+	+	−
7	< 2300	60	0·60	+	+	+	−

(+) Presence of the antigenic determinant.
(−) Absence of the antigenic determinant.

It is significant that the small mol. wt. band is consistently found in the same position on 15% SDS gels, at a molecular weight of about 3800, whether the initial material or the Sephacryl fractionated material was assayed (Fig. 3). This argues in favour of the 3800 fragment being a constant and possibly smallest fragment of SA I/II.

The carbohydrate content of the 3800 mol. wt. material was between 1·64 and 2·08%, in contrast to 6·57 (±1·63)% in the starting 185,000 mol. wt. material. The carbohydrate content of the 3800 SA I/II must be therefore negligible, as 2% would allow for carbohydrate corresponding to a mol. wt. of only 80. The lipid content was determined by thin layer

Table 3. The R_f values of five lipids detected by thin layer chromatography in the 3800 and 185,000 streptococcal antigen preparations

Markers	R_f Values	% R_f	% R_f values of samples 1 185,000	2 185,000	3 185,000	4 3800
Solvent front	16/16 = 1·00	100				
1 Phospholipid	0/16 = 0·00	0	0	0	0	0
2 Cholesterol	1·5/16 = 0·09	9	—	—	—	—
3 Free fatty acid	5/16 = 0·32	32	28	28	28	—
4 Triglyceride	9/16 = 0·56	56	55	55	57	—
5 Cholesterol ester	13·5/16 = 0·85	85	89	90	90	—

(—) Indicates no detectable lipids.

chromatography and the 3800 material failed to show any detectable lipids, unlike the 185,000 material. We conclude then, that the 3800 purified material, containing streptococcal antigen I and II determinants, is a protein, with no lipids and only a trace of carbohydrates. The biological properties of this small mol. wt. antigen are now being subjected to extensive investigations.

REFERENCES

Carlsson J., Newbrun E. & Krasse B. (1969) Purification and properties of dextran-sucrase from *Streptococcus sanguis*. *Archs oral Biol.* **14,** 469.

Clamp J.R. (1974) Analysis of glycoproteins. *Biochem. Soc. Symp.* **40,** 3.

Folch J., Lees M. & Stanley G.H.S. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. biol Chem.* **226,** 497.

Gray W.R. (1972) End-group analyses using dansylchloride. *Meth. Enzymol.* **25,** 121.

Guggenheim B. & Newbrun E. (1969) Extracellular glucosyltransferase activity of an HS strain of *Streptococcus mutans*. *Helv. Odontol. Acta.* **13,** 84.

Hamada S. & Slade H.D. (1980) Biology, immunology and cariogenicity of *Streptococcus mutans*. *Microbiol. Rev.* **44,** 331.

Hardy L., Jacques N.A., Forester J., Campbell L.K., Knox K.W. & Wicken A.J. (1981) Effect of fructose and other carbohydrates on the surface properties, lipoteichoic acid production, and extracellular proteins of *Streptococcus mutans* Ingbritt grown in continuous culture. *Infect. Immun.* **31,** 78.

Kaulen H.D. (1972) Separation of phosphatidylserine and phosphatidylinositol by one-dimensional thin-layer chromatography of lipid extracts. *Analyt. Biochem.* **45,** 664.

Lowry O.H., Rosebrough N.J., Farr A.L. & Randall R.J. (1951) Protein measurement with the folin phenol reagent. *J. biol. Chem.* **193,** 265.

McCabe M.M., Hamelik R.M. & Smith E.E. (1977) Purification of dextran-binding protein from cariogenic *Streptococcus mutans*. *Biochem. biophys. Res. Commun.* **78,** 273.

Russell M.W. (1979) Purification and properties of a protein surface antigen of *Streptococcus mutans*. *Microbios.* 7.

Russell M.W., Bergmeier L.A., Zanders E.D. & Lehner T. (1980a) Protein antigens of *Streptococcus mutans*: Purification and properties of a double antigen and its protease-resistant component. *Infect. Immun.* **28,** 486.

Russell M.W. & Lehner T. (1978) Characterisation of antigens extracted from cells and culture fluids of *Streptococcus mutans* serotype c. *Archs oral Biol.* **23,** 7.

Russell M.W., Zanders E.D., Bergmeier L.A. & Lehner T. (1980b) Affinity purification and characterization of protease-susceptible antigen I of *Streptococcus mutans*. *Infect. Immun.* **29,** 999.

Russell R.R.B. (1979) Wall-associated protein antigens of *Streptococcus mutans*. *J. gen. Microbiol.* **114,** 109.

Smith R. & Lehner T. (1981) A radioimmunoassay for serum and gingival crevicular fluid antibodies to a purified protein of *Streptococcus mutans*. *Clin. exp. Immunol.* **43,** 417.

Zanders E.D. & Lehner T. (1981) Separation and characterization of a protein antigen from cells of *Streptococcus mutans*. *J. gen. Microbiol.* **122,** 1.